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New insights into skin structure: scratching the surface

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Abstract

The formation, structural organization, and barrier functions of stratum corneum (SC) are reviewed. Stratum corneum is considered as a composite material and a biopolymer with properties so unique as to consider it a 'smart material'. SC, together with stratum granulosum (SG) responds (as an actively smart material) to environmental signals with appropriate modulations in its barrier properties. Current theories on the mode of barrier formation, validity of use of animal models and ex vivo human skin in studies of percutaneous absorption, as well as its implications in development of transdermal systems (TDS) are discussed. Potential pitfalls in extrapolating from animal data and the use of cadaver skin/epidermal membranes in evaluations of TDS are also stressed.

Keywords: Stratum corneum; Lamellar bodies; Ultrastructure; Permeability barrier; Transepidermal water loss; Smart materials; Transdermal delivery systems

Contents

1. Introduction

The integument, especially that of vertebrates, is a marvelous organ system. It is not only the largest organ in the body, but also perhaps the most complex, with at least five different cell types

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contributing to its structure, and other cell types from circulatory and immune systems being transient residents of the skin. In terms of the number of functions performed, the skin simply outweighs any other organ: its primary function is of course protection, which covers physical, chemical, immune, pathogen, UV radiation and free radical defenses. It also is a major participant in thermoregulation, it functions as a sensory organ, performs endocrine functions (Vitamin D synthesis, peripheral conversion of prohormones), is significant in reproduction (secondary sexual characteristics, pheromone production), and perpetuation of the species, human non-verbal communications (visual signaling, emotions expressed), as well as a factor in zenophobia and bias against fellow humans that has shaped the destiny of humanity. Skin is also the basis of several billion-dollar industries such as personal care, cosmetics, and fashion businesses. For the pharmaceuticals, it is both a challenge (barrier) and an opportunity (large surface) for delivering drugs. The reader is directed to a very recent discussion forum on 'What is the True Function of Skin' [1] to explore the many fascinating facets of skin functions. Additionally, the use of animal models, bioengineered skin equivalents, or skin obtained from surgery or cadaver skin for skin penetration and bioequivalence studies have raised debates about what is the most appropriate model for such evaluations, and the validity of the use of isolated sheets of stratum corneum by physical scientists as a 'model membrane'. This brief introductory statement underlines the fact that it is impossible for an individual to have good insights into all of skin's form and functions and even a superficial compilation logically cannot be condensed into one chapter. For practical reasons, this review will be limited to the epidermal permeability barrier: the stratum corneum and the cells that generate it, which is just 'scratching the surface'.

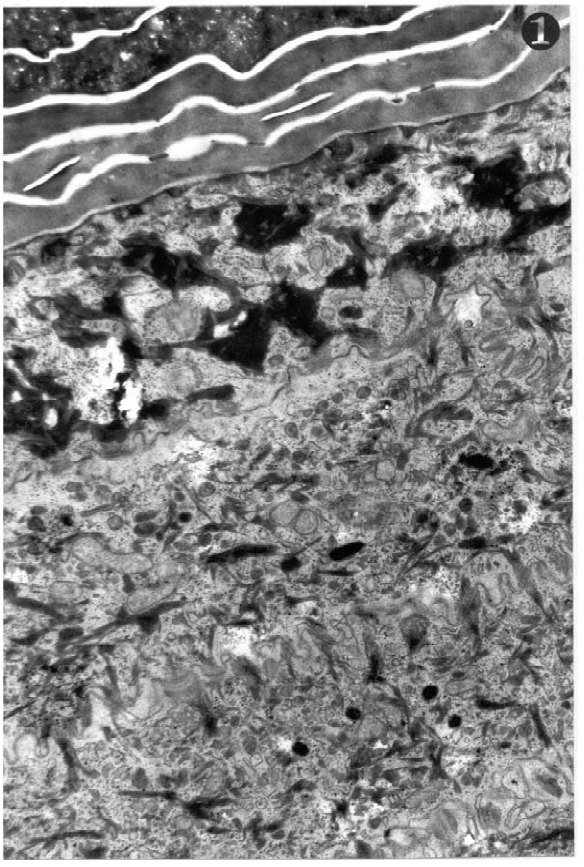

Fig. 1. Low magnification electron micrograph of human epidermis, showing upper stratum spinosum (SS), stratum granulosum (SG), and part of stratum corneum (SC). Note lamellar bodies (arrows) and keratohyalin granules (KHG). SC intercellular domains containing lamellar lipids appear empty, and are seen only following RUO4 fixation.

2. Histology of the mammalian skin

The skin consists of two distinct layers. The dermis (forming the bulk of skin) is made up of connective tissue elements. The overlying, avascular epidermis is composed primarily of keratinocytes (Fig. 1). Dermis is made up of collagen, elastin, glycosaminoglycans, collectively termed the extracellular matrix (ECM), as well as fibroblasts that elaborate the ECM. Dermis is highly vascular, and also includes the pilosebaceous units, sweat glands, dermal adipose cells, mast cells, and infiltrating leucocytes. About 95% of the epidermis layer is constituted by keratinocytes (of which the lowermost are anchored to the basement membrane via hemidesmosomes), and the rest are melanocytes, Langerhans cells, and Merkel cells (mechanoreceptors). The stratified epidermis, ~100 to 150 μm thick, is divisible into four distinct layers: the

stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG), and stratum corneum (SC).

2.1. Stratum basale (SB)

The stratum basale is a single layer of columnar basal cells, which remain attached to the basement membrane via hemidesmosomes. The SB is composed of epidermal stem cells and transiently amplifying cells derived from them. The cells show a high nucleo-cytoplasmic ratio, cell organelles such as mitochondria, and keratin filaments (tonofilaments) that are inserted into the hemidesmosomes. Desmosomes connect adjacent and overlying cells. Two keratins, K14 and K5, are expressed in the basal cells.

2.2. Stratum spinosum (SS)

Due to the abundance of desmosomes, this layer has a spiny appearance of its cells in histological sections. In addition to the typical cell organelles seen in the basal layer, the SS also shows the presence of lipid-enriched lamellar bodies (LB) that have been called Odland bodies, keratinosomes, membrane-coating granules that first appear in this layer. Ultrastructurally, LBs are 0.2 to 0.5 μm in diameter, with parallel stacks of lipid-enriched disks enclosed by a trilaminar membrane. In near perfect cross sections, each lamellae shows a major electron dense band that is shared by electron lucent material divided centrally by a minor electron dense band (Fig. 2, inset). Their appearance marks the dual aspects of epidermal differentiation, viz., protein and lipid synthesis. An increase in cellular keratin filaments is noticeable compared to the basal cells. Keratins 1 and 10 are the biochemical markers for this layer. In the upper layers of SS, the cells begin to flatten and elongate, and grade to the stratum granulosum.

2.3. Stratum granulosum (SG)

Distinct, darkly staining keratohyalin granules (KHG), characterize this layer in histological preparations. KHGs are composed of profillaggrin, loricrin, and a cysteine-rich protein as well as keratins 1 and 10, and they become progressively larger in the upper granulocytes (Fig. 1), reflecting a quantitative increase in keratin synthesis. The fillagrin subunits of profillagrin play the role of matrix molecule to aggregate and align the keratin filaments. Keratin filaments in upper granular layers are highly phosphorylated and have extensive disulfide bonds, compared to the cell layers below. The progressive cell differentiation, evident in increased protein synthesis, is also accompanied by an increased lipogenesis (i.e. presence of large numbers of lamellar bodies), which reach their highest density in the uppermost granulocytes (about 20% of the cell cytosol). The uppermost cells in the SG display a unique structural and functional organization of the lamellar bodies, consistent with their readiness to terminally differentiate into a corneocyte, during which the lamellar bodies are secreted to the extracellular domains (Fig. 2a). The role of LB-derived lipids in formation of the permeability barrier is illustrated in Fig. 2b, where colloidal lanthanum, an electron dense tracer, injected subcutaneously is seen to move outwards through the intercellular space of epidermis, but the efflux is stopped at the level where the secreted LB contents fill the SG–SC interface. As seen in electron micrographs of oblique sections, LBs are highly polarized in the apical cytosol of upper granulocytes. Investigations employing tools such as confocal scanning and electron microscopy and techniques like enzyme cytochemistry [2] show lamellar bodies budding off a transgolgi-like network and remain interconnected within the secretory granulocyte. LBs have also been isolated and their biochemical composition, which includes glucosyl ceramides, phospholipids, cholesterol, and hydrolytic enzymes like lipases, sphingomyelinase, β-glucosylcerebrosidase and phosphodiesterases characterized. There is ample evidence that once secreted, their lipid contents are processed by the co-secreted enzymes, transforming the morphologically short stacks of probarrier lipids into the ceramide-enriched final barrier lipid structures [3].

Electron microscopy also suggests that the disk structures within individual lamellar bodies are already continuous, having an accordion-like folded pattern, and that these contents unfurl on secretion [4]. However, whatever form the disks are within the LBs, further fusion of the secreted contents mediated by co-secreted enzymes and/or fusogenic lipids

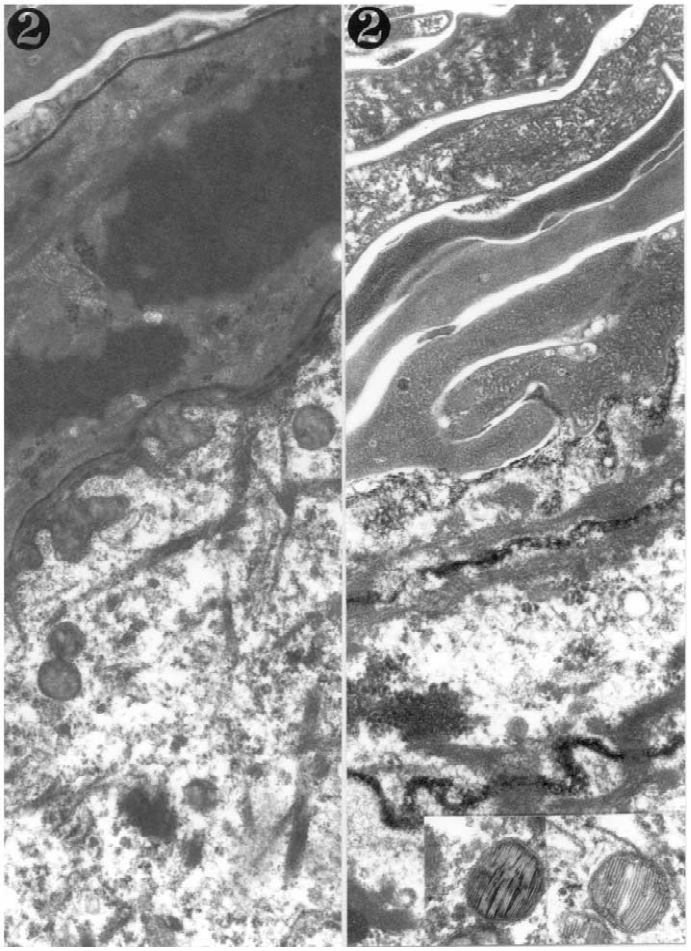

Fig. 2. (a) Higher magnification view of part of SG, and a transitional cell (halfway into being cornified). Note the lamellar bodies (LB) poised to secrete, as well as invaginations of apical SG membrane, site of LB secretion. Modified from Ref. [37] with permission from Springer. (b) Colloidal lanthanum, a water soluble tracer, permeating outwards (arrows) following subcutaneous injection, stops where secreted LB contents fill the SG–SC interface. A visual demonstration of the site of permeability barrier. Inset: lamellar bodies, post fixation with RUO4 (left) and OSO4 (right).

formed due to enzyme activity (lysophospholipids) result in formation of the SC extracellular bilayers. The initiation of the formation of cornified envelope, the large scale secretion of lamellar bodies, the dissolution of the cellular organelles, the condensation of keratin filaments, etc. that lead to the irreversible process of cornification, depend on many signals, the nature of which is still being elucidated. One such signal that triggers the process is ionic calcium. In vitro studies have shown that keratinocyte differentiation can be induced by elevating the calcium concentration of their culture media [5]. Cytochemical techniques [6] as well as particle beam analysis have demonstrated an extracellular calcium gradient in mammalian epidermis in vivo, with low Ca^{2+} content in the basal, proliferating layers, and progressively higher concentrations as the epidermis stratifies and differentiates. An influx of Ca^{2+} into the cytosol of upper granulocytes is believed to trigger the rapid transformation of granulocyte into a corneocyte. Via an intermediate stage, the transitional cell characterized by remnants of nuclei and other cytosolic components, unidentifiable vacuoles, dense, keratin-filled cytosol and cornified

cell envelope. This process of transition from granular to the first cornified cell is rapid (5 to 6 h) and the mechanism of cytoplasmic degradation involving activation of several proteases, despite absence of morphological evidence of autolysosomes, is poorly understood [7].

3. The stratum corneum (SC)

The stratum corneum (SC) is a composite of the corneocytes (terminally differentiated keratinocyte) and the secreted contents of the lamellar bodies (elaborated by the keratinocytes), that give it a brick-and-mortar organization [8] This arrangement creates a tortuous path through which substances have to traverse in order to cross the SC. In human skin, stratum corneum typically has about 18 to 21 cell layers. Individual corneocytes are 20 to 40 μm in diameter (as opposed to 6 or 8 μm for the basal cell). They may differ in their thickness, packing of keratin filaments, number of desmosomes (corneodesmosomes), etc., depending on the body site and their location within the SC (inner stratum compactum vs. outer stratum disjunctum). These features also may influence their degree of hydration, which varies from 10 to 30% bound water. Water-holding properties of corneocytes are influenced by the rate of proteolysis (fillagrin breakdown), leading to formation of a blend of amino acids termed as natural moisturizing factors [9]. The ridges and undulations of corneocytes aid the overlapping cells to interdigitate, enhancing the stability of the layer. In addition, corneodesmosomes ensure the cohesiveness of the layer, especially in the stratum compactum, where they appear intact (Fig. 3). In contrast, slow degradation of these structures in the stratum disjunctum allows the normal process of corneocyte desquamation.

The ultrastructural appearance of RUO4-stained SC provides a good appreciation of the mode of sequestration of lipids as membrane bilayers within the SC extracellular domains. These static images in electron micrographs provide a glimpse, albeit a frozen moment, of the dynamic post-secretory modulations in the probarrier lipids (Fig. 4). Whereas the

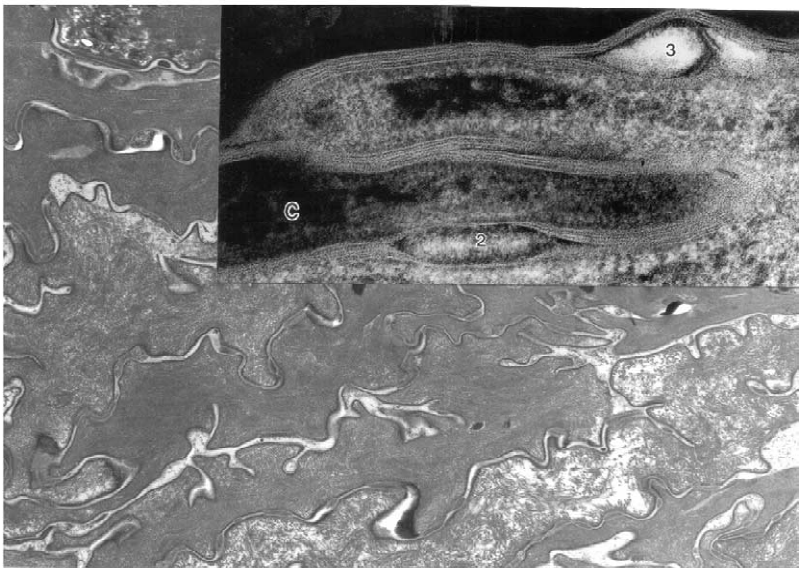

Fig. 3. Electron micrograph of human SC showing the highly interdigitating nature of adjacent and overlying corneocytes (bricks), as well as the high degree of tortuosity of the extracellular pathway (mortar). Inset: when post fixed with RUO4, the mortar lipids of murine SC are visualized as lamellar structures spanning the SC extracellular space except regions punctuated by desmosomes. Two stages in desmosomal dissolution are shown: (2) advanced state of degradation, and (3) ballooning into a lacuna embedded within the lipid bilayers. C, corneocytes. Reproduced from Ref. [3] with permission from John Wiley.

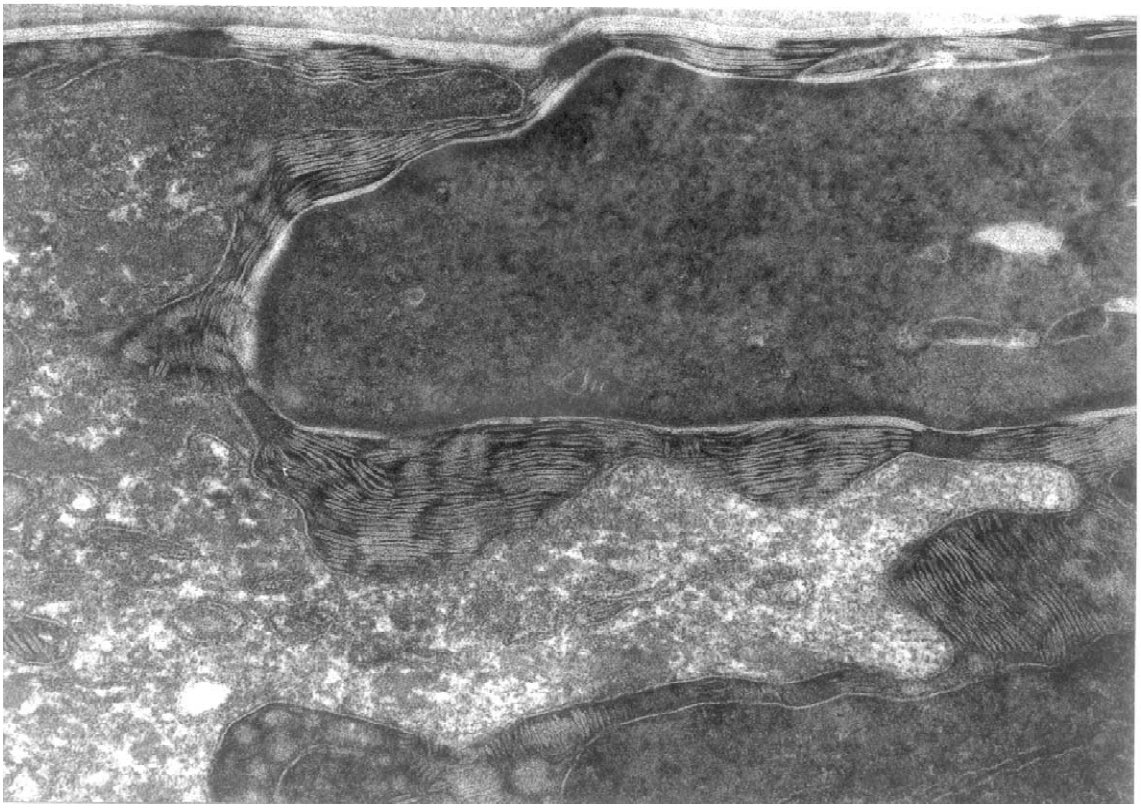

Fig. 4. SG–SC junction of murine epidermis following RUO4 post fixation shows post secretory processing of LB-derived disks, such as end-to-end fusion and transformation into broad, compact bilayers at the distal portions, anchoring of the disks to intact desmosomes, etc. Reproduced from Ref. [3] with permission from John Wiley.

proximal parts of the SG–SC interface contain separate disks or those in the process of fusing with each other, close to the membrane of the first corneocyte, the fused LB disks have already formed continuous lipid bilayers. The basic unit pattern of the bilayers consists of a series of six electron-lucent lamellae alternating with five electron-dense lamellae. Double and triple basic units occur frequently. The basic unit structures persist all the way to the outermost layer of SC, although contamination with sebum results in loss of the tight arrays of bilayers. Additionally, the structural relation of the bilayers to the corneodesmosomes shows gradual changes associated with the progressive degradation of desmosomal structures. Ultrastructurally, the process of desmosomal breakdown involves: (1) the formation of electron-lucent areas in their core and (2) eventual expansion or ballooning of the cores to form the lacunar domains, which are gradually engulfed by the extracellular bilayers (Fig. 3, inset). The near total segregation of lipids to SC extracellular domains was also confirmed by isolating SC membrane sandwiches containing trapped intercellular lipids [10]. These preparations comprised about 50% lipids by weight, accounting for over 80% of SC lipids, and had the same lipid profile of whole SC. Additionally, it had the same freeze fracture and X-ray diffraction pattern of whole SC. Ceramides, cholesterol, and fatty acids are present in roughly equimolar ratios, in addition to small amounts of triglycerides, glycosphingolipids, and cholesterol sulfate that are detected in the SC [11]. Ceramides amount to ~50% of the total lipid mass and 40% of the total number of lipids, and are very crucial to the lipid organization of the SC barrier [12]. Of the seven major ceramide classes, ceramide 1 is believed to be

uniquely significant in the formation of the covalently bound lipid envelope of corneocytes. Ceramide 1 consists of sphingosine and long chain unsaturated, mono- and di-unsaturated omega hydroxy acids in the amide linkage. Cholesterol is the second most abundant lipid in the SC and amounts to ~25% weight or 30 mol.% of SC and is crucial for promoting the intermixing of different lipid species. Free fatty acids account for about 10% of SC lipids or 15 mol.%, and consist predominantly of long-chain saturated fatty acids having more than 20 carbon atoms. Oleic (6%) and linoleic (2%) are the only unsaturated fatty acids detected as free in the SC [11].

Deficiencies in any one of these three lipid species result in barrier abnormalities characterized by increased trans-epidermal water loss (TEWL) as well as observable alterations in the ultrastructural features of the SC extracellular domains [3]. These abnormalities could arise from experimental inhibition of key epidermal enzymes involved in synthesis of cholesterol (HMG Co A reductase), glycolipid synthesis (serine palmitoyl transferase), fatty acid synthesis (fatty acyl co carboxylase), or in extracellular processing of glycolipids that are secreted as pro-barrier lipids via the lamellar body secretory system (β-glucocerebrosidase). These barrier defects also lead to epidermal hyperproliferation, as well as dry, flaky skin conditions. Epidermal sterologenesis has been shown to be independent of circulating levels of cholesterol and hence systemic cholesterol-lowering drugs do not usually impact the epidermal barrier.

4. The organization of stratum corneum

The classic brick and mortar organization, championed by Peter Elias, is still the most simplistic organizational description. The protein-enriched corneocytes (bricks) impart a high degree of tortuosity to the path of water or any other molecule that traverses the SC, while the hydrophobic lipids, organized into tight lamellar structures (mortar), provides a water-tight barrier property to the already tortuous route of permeation in the interfollicular domains (a shunt pathway, or follicular route of penetration does exist in the skin, and is not part of this discussion). The corneocytes impart physical protection (e.g. impact resistance), as is evident from studies on the gene gun [13] where most of the gold particles shot through a gene gun at 500 p.s.i. remained embedded within the SC cells, and only a few reaching the nucleated layers and the dermis. The orientation of the keratin filaments could be a factor in lateral propagation of the stress (of impact), and through the desmosomes to the adjacent corneocytes. The exceptionally large numbers of desmosomes seen in the SC of Rhinos is perhaps not accidental.

In the lipocentric world of barrier research, the mortar lipid has received more attention than the corneocytes. While the foregoing section concerns its composition, several theories have been forwarded to address its structural organization at a micro- and molecular level. The lipids were considered to be in a liquid crystalline [14], a blend of liquid crystalline and gel phases [15,16] or a single coherent gel phase [17]. The domain mosaic model of Forslind [15] predicted that the bulk of gel lipids have grain boundaries that are in liquid crystalline state, and that water passes through this meandering route, which imparts a further degree of tortuosity to an already tortuous macro structure of the SC. The ‘plastic crystalline’ model of Norlen [17] predicts a single coherent gel, with no phase separations. The reader is directed to a discussion by Bouwstra and Ponec [18] and Norlen [19] on the pros and cons of this theory. Irrespective of the physical organization of the SC lipids, existence of a pore-pathway through the SC for hydrophilic molecules has long been debated [20]. The rapid delivery of large, hydrophilic molecules that has been achieved using physical means of drug delivery (iontophoresis, ultrasound, photomechanical stress waves, etc.), without disrupting the entire lipid organization, prompted Menon and Elias [21] to investigate the morphological basis of such a pathway. Using electron-dense tracers, with the various modes of delivery, it was found that the tracers usually accumulated within the ‘lacunae’, electron-lucent spaces within the bilayers, possibly having their origin in degrading desmosomes. The proposed pathway is a transient channel formed by continuities between the lacunae under different modalities of drug delivery, such as iontophoresis, sonophoresis, and photomechanical pressure waves.

It is also proposed that once the devices are turned off, the 'pore pathway' collapses back into the original, discontinuous lacunar structures. Under this scenario, the SC is not an unyielding, rigid brick wall, but rather a composite biopolymer, responding to forces with appropriate deformations in its structural organization. A composite structure is made up of two or more dissimilar materials, which when combined are better (meaning that they are tougher, stronger, and more durable) than each would be separately (i.e. gains complex properties). The stiff (bricks) and compliant (mortar) phases of SC combined in parallel, achieve stress concentration and interesting barrier phenomena due to interfacial effects (the covalently bound lipid envelope of the corneocyte). Composites have been considered high tech developments, but to a large extent they are biomimetics. The morphology of SC has an uncanny resemblance to the structural organization of stainless steel, a pseudocomposite (Fig. 1 in Ref. [22]). But with its high flexibility, SC can behave as a biopolymer or a membrane. It is easy to see why experimental studies on skin penetration employ an isolated sheet of SC sandwiched between diffusion cells or similar contraptions: this 'membrane' is a barrier to be overcome for transdermal delivery of drugs or other chemicals. However, taking a holistic view of the structure and functions of SC reveals that the 'living stratum corneum' (Fig. 5) manifests autopoesis (i.e. maintains its defining organization through experimental perturbations and changes, regenerating its components in the course of this operation) [23]. The stratum corneum replenishes both the barrier lipids and its scaffolding, the corneocytes. It is a self-cleansing system in that its microflora is cleansed off by exfoliation as well as antimicrobials such as defensins and sphingosine, both products of keratinocytes. SC senses ambient humidity and generates natural moisturizing factors in response to decreased humidity by activating

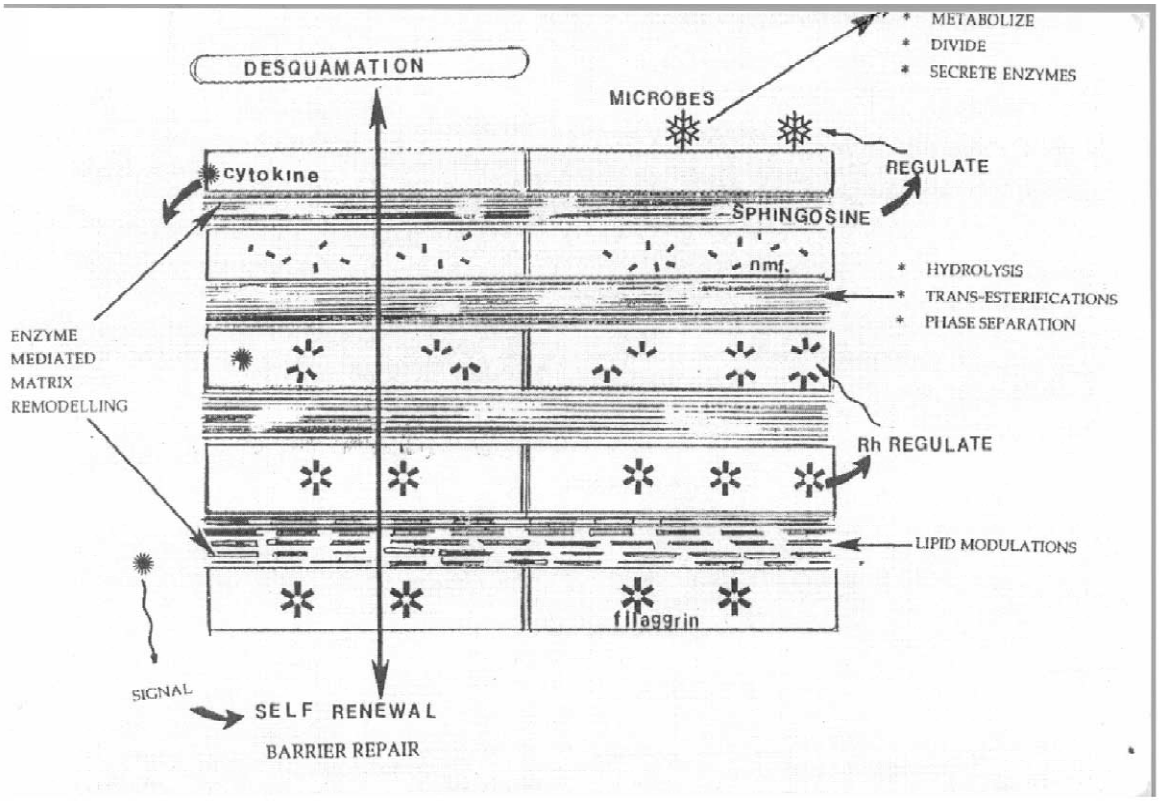

Fig. 5. Diagrammatic representation of the holistic view of SC.

Table 1
Attributes of a smart system

Passively smart (SC)	Actively smart (SG–SC)
Selectivity	
Shapeability	Senses ambient changes
Self-recovery	Uses feedback system
Simplicity	Makes a useful response
Self-repair	
Stability	

Table 2
Events upregulated for barrier repair

Nascent LB secretion
Ionic and cytokine signaling
Lipid synthesis (cholesterol, FA, ceramides)
New LB formation and secretion
Post-secretory processing of LB contents
DNA synthesis

proteases. In short, it functions as a 'smart system'. Smart systems, in the materials world, are classified as 'passively smart' and 'actively smart' [24]. Table 1 shows the passively smart responses of an isolated SC membrane and actively smart attributes of the stratum corneum in vivo (i.e. with an intact SC–SG interface). The distinguishing feature of the latter is its ability to sense ambient changes, use a feedback system, and make a useful response. In this role, SC is truly the body's interface with the rest of the world: it is the organ of touch, where signals are received, signals are sent, emotions are expressed, and dysfunctions are diagnosed. One such actively smart response is the barrier repair phenomena. The dramatic increase in TEWL following removal of lipids (solvent extraction) or corneocytes and lipids (tape stripping), sets in motion a series of signals (ionic, cytokines, etc.) that have been well documented to initiate cellular and metabolic responses in the underlying nucleated epidermis (Table 2), leading to replenishment of barrier lipids and restoration of the permeability barrier. The time course of the barrier repair response can be followed non-invasively by monitoring the TEWL, which is a measure of barrier integrity.

5. The site of action

Whether for initial formation of barrier, or its repair and homeostasis, the SG–SC interface (Fig. 4) is the site of action. What is seen in Fig. 4 is a frozen moment in the life of epidermis: LBs are secreted, their disk contents unfurl, realign, and fill the extracellular space. The disk contents anchor onto desmosomes, fuse end-to-end with each other and go through the enzyme catalyzed lipid processing that converts the 'probarrier' lipids into the final lamellar structures that make up the unique blend of mortar lipids, enriched in ceramides, cholesterol and free fatty acids. The unique arrangement of the lamellar body secretory system within the secretory granular cell may explain the ability for rapid LB secretion to support the homeostasis and/or rapid repair of the permeability barrier [3]. Most significantly, continuities between the extracellular domains of the SG–SC interface with deep, interdigitating invaginations form an extensive honey-comb like latticework within the apical cytosol of the uppermost SG cell seen in oblique sections of skin (Fig. 6). This organization provides for portals of LB secretion as the granulocyte elaborates a thickened envelope, the cornified envelope, in preparation for its final transition to a corneocyte. The cornified envelope is a thickened, electron-dense band (as seen in electron micrographs) underlying the apical plasma membrane. The thickening represents the sequential deposition of proteins, cross linked by (glutamyl) lysine isopeptide linkages, bis(glutamyl) polyamine linkage and disulfide bonds [4]. The cross-linking is catalyzed by transglutaminases, whose major substrate is involucrin. Loricrin, the major structural protein of the envelope, is incorporated at a relatively late stage. Other putative constituents of the envelope are cornifin, where the outermost SG cell is replaced by a solvent-resistant envelope [25]. This structure is enriched in omega hydroxyceramides, covalently bound through alternating and C-1 sphingosine hydroxyl groups to peptides in the outer cornified envelope (primarily glutamine/glutamic acid residues in involucrin). The origin of this structure is uncertain (lamellar contents, limiting envelope of sphingomyelin residues in the plasma membrane, etc.). The last two options would allow transglutaminase 1, the calcium-dependent enzyme required for cornified envelope cross-linking, to transesterify hydroxyceramides in situ. The function of

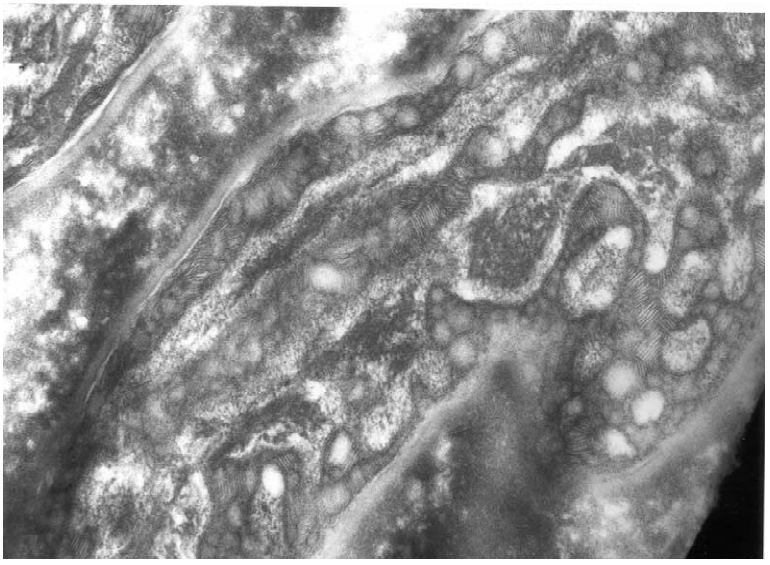

Fig. 6. Oblique section of SG–SC interface from murine epidermis showing the highly convoluted nature of this junction which is not apparent in near-perfect cross sections (such as in Fig. 2).

the covalently bound envelope, whether it is required for barrier function, corneocyte cohesion, and/or regulation of access/egress of molecules from the corneocyte cytosol, is uncertain. The near perfect cross section shown in Fig. 4, however, does not reveal the truly convoluted nature of this domain, which can be appreciated in slightly obliquely cut sections. Fig. 6 demonstrates the complex interdigitating interface, with deep invaginations of SG that serve as portals for LB secretion. A battery of techniques using EM and laser confocal microscopy [2] has unraveled the 'lamellar body secretory system', a trans-golgi-like tubulo-reticular network connecting several LBs that appear to bud off from the system. This arrangement is considered to provide precise control for barrier maintenance and homeostasis. Opposed to this model is the recently proposed membrane unfolding model of Norlen [17]. He proposes that LBs are artifacts of electron microscopy, and that barrier lipids exist in the cubic phase within the SG, and as it is secreted at the SG–SC interface, transform into lamellar structures on dehydration. This model is based on lipid biophysics as well as logical consideration of what is energetically the most favorable means of transporting lipids to the extracellular space and transforming it into a lamellar organization. However, a wealth of available experimental data and evidence argue against this model. These include: (1) isolation of discrete lamellar bodies from epidermal preparations; in much the same way as other cellular organelles such as mitochondria are isolated; (2) the barrier abnormality in Gaucher's disease, where incomplete processing of secreted lipids due to absence of a single enzyme, β-glucocerebrosidase, results in abnormal lamellar structures with accompanying elevated trans-epidermal water loss; and (3) similar defects in lipid organization resulting from topically applied inhibitors of enzymes that are key to lipid processing. Lastly, illustrations of cubic lipid structures in other cell types that were used by Norlen to support his theory have been obtained by the very same EM techniques that have revealed lamellar bodies rather than cubic structures in the epidermis. It is improbable that the same protocol of tissue processing for electron microscopy would create artifacts in epidermis, but not in other cell types.

Notwithstanding the model of barrier formation, barrier homeostasis (i.e. repair and maintenance of the barrier after it has been breached) should provide insights into development of transdermal drug delivery systems. The barrier repair response of skin

would work against effective delivery of drugs from a transdermal system over a period of time. Two of the pertinent issues to consider here are: (1) how well can we extrapolate from barrier repair studies in animal models, and (2) how reliable/predictable are results obtained from experimental work with ex-vivo or cadaver skin.

Comparison of the morphology of murine and human skin reveal that the SG–SC junction in human skin is not as replete with secreted LB contents as in the former (Fig. 7). The other obvious differences are in the number of cell layers in SC: there are 16 to 18 layers in human skin vs. 6 to 7 layers in mouse skin. Yet, the basal TEWL rates are more or less comparable, as could be gleaned from the literature. The barrier repair time (to regain basal values after experimental barrier disruption) is shorter in murine models (24 h) than in humans (60–80 h) (Table 3). At first glance, it would appear that the murine system is more efficient than humans, but this could be interpreted in two ways. Firstly, the barrier homeostasis in humans is not as robust as that in murine, due to the less critical nature of skin barrier for survival in humans (survival of premature babies and burn victims are cases in point). Humans have practically absolute control over their environment by clothing (microenvironment) as well as humidity-, temperature-, and radiation-controlled

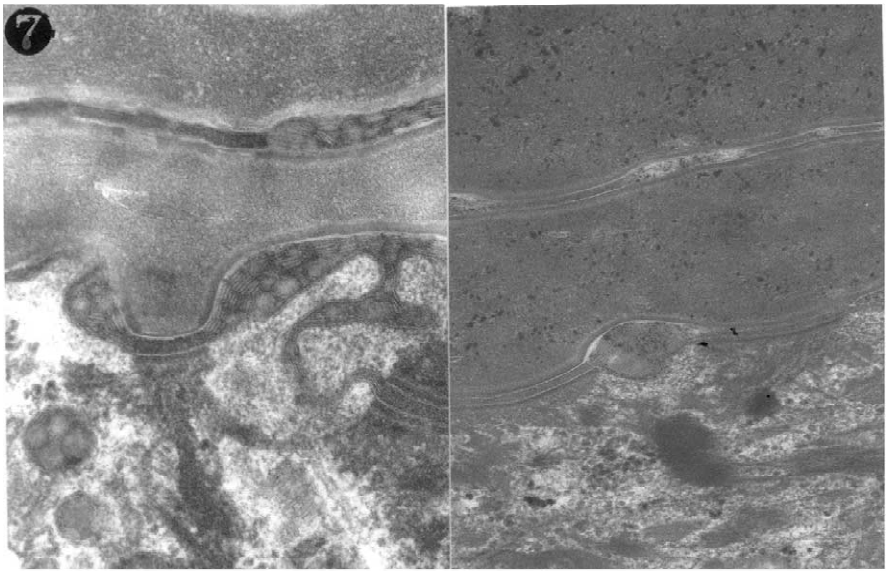

Fig. 7. Comparison of murine (left) with human (right) SG–SC domains shows a less prominent LB secretion in the latter.

Table 3
Murine vs. human: differences in barrier function and homeostasis

Barrier	Murine	Men
SC	Thin SC	Thick SC
SC–SG interface	Packed LB disks	Fewer LB disks
LB secretion	Significant	Fewer
Barrier	Not so robust	Robust
Disruption	Less time, easier	Longer, harder
Repair	24 h	Takes longer (60–70 h)
Affected surface	Significant % of body	Negligible % of body
Microenvironment	Relatively unmodified (except lab climate)	Highly modified and controlled

transportation and housing (macroenvironment). This of course comes with a price, and the general perception of increasing incidence of sensitive skin and atopy may be related to changing habits in skin care and even improvements in housing conditions. A recent report by Cork et al. [26] reported data for England over the past 30 years or so, showing that the average frequency of bathing the babies was up from 2.5 times to 7 times per week. The report also shows a dramatic increase in sale figures of bath and shower products, home water usage, the numbers of conventional hard wood floors being replaced by carpets, and installation of central heating (the last two being conducive to higher incidence of dust mite infestations pivotal to developing atopic dermatitis). These are pointers to how Homo sapiens, an over-specialized species, are eroding the defenses of the integumentary system. Add to this the knowledge that stress, including psychological [27], affects the permeability barrier homeostasis and could exacerbate skin dysfunctions. On the other hand, the reported differences between man and mouse could simply be a reflection of vast differences in experimental design: most experiments involved a significant area of the body surface in mice but a negligible surface area in humans, and if there is a threshold for either the surface area or TEWL needed to trigger the repair response, this could skew the results, making extrapolation truly unreliable. The implication for research into transdermal delivery systems is that if drug delivery patches or other devices remain under such a threshold, the 'window' would remain open for a reasonable duration of time, without eliciting the barrier repair response that works against it. This benefit could be further augmented by employing metabolic intervention strategies as discussed by Elias et al. [28].

6. Relevance of ex-vivo skin

Use of ex-vivo human skin or cadaver skin is a more attractive alternative, and logical for the research aimed at developing transdermal delivery systems for therapeutics. Let us examine how biologically sound this approach is. Many of the skin penetration studies employ isolated stratum corneum that is often from freezer-stored, subsequently thawed cadaver samples, or heat- or enzyme-separated from samples obtained from cosmetic surgery, sandwiched in a Franz cell or a similar system, where the SC 'membrane' remains fully hydrated for several hours or days. The effects of hydration on the stratum corneum has been well documented, and several studies have shown a near total disruption of the lipid bilayers, 'roll-up' of the lamellae [29] or formation of extensive vesicles in the SC extracellular domains [30]. Gene delivery by biolistic process (gene gun) has also been evaluated in ex-vivo human skin from surgery [13], and even when maintained for 24 h in culture medium, the disrupted mortar lipids were left unrepaired. This indicates that even when full thickness skin is used, the 'smart response' of the skin is completely obliterated, and that the barrier homeostatic mechanism is no more functional ex vivo. Using cadaver skin has inherent drawbacks, such as potential freeze–thaw damage to the SC extracellular lipids, and definitely to the nucleated epidermal layers. These structural alterations can be appreciated from Fig. 8, showing disorganization of the secreted LB contents, and lateral expansions of the lacunar domains. Attempts to permeabilize cadaver skin using photomechanical pressure waves were unsuccessful, as opposed to successful skin permeabilization and drug delivery in vivo using this technology (Doukas, personal communication). These are pointers to the fact that prolonged storage and/or freeze–thawing of skin alters not only the SC barrier organization, but the functional properties of the tissue as well. Despite a recent publication that TEWL does not correlate with skin barrier function in vitro [31], TEWL is a true reflection of the barrier status in vivo when measured properly. In their experiments, Chilcott et al. [31] used skin stored at $-25\,^{\circ}C$ (for a maximum of 14 days), followed by thawing and heat separation of the epidermis. Again, full thickness skin or the 'epidermal membranes' treated in this way were fully hydrated in Franz-type diffusion cells for evaluating flux of labeled water. Any conclusions on the relationship between TEWL and skin permeability drawn from such studies is fraught with the peril of ignoring altered properties of the SC, not to mention the absence of biological responses of the integumentary system.

In contrast, skin responses in vivo could be very different: prolonged hydration damage to SC lipid

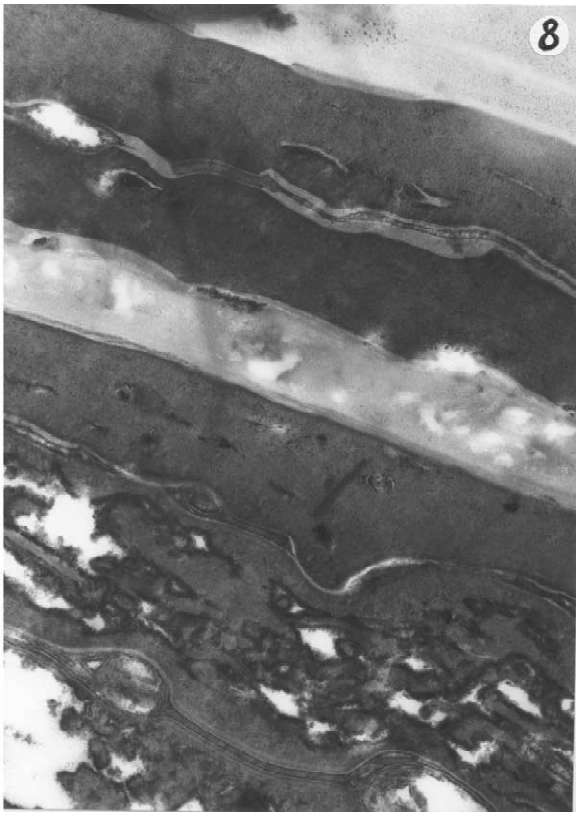

Fig. 8. Electron micrograph of cadaver skin showing structural alterations in barrier lipid organization.

lamellae may trigger enhanced LB secretion at the SG–SC interface, and possibly modified lipid processing, so as to adapt the barrier lipids to become more hydration compatible. It is known that marine mammals retain glucosylated lipids in the SC lipids and lack the tight bilayers characteristic of terrestrial mammals, yet have large numbers of LBs that are actively secreted [32]. Whether strategies mimicking that of marine mammals will come into play in adaptation to prolonged hydration in terrestrial mammals has not yet been evaluated.

7. The holistic barrier response

It is fairly well known that a patch test or a UV insult to skin elicits an acute depletion of Langerhans cells from the epidermal compartment, and in a dark skinned individual (phototype 3 or 4), a post-inflammatory hyperpigmentation is evident days later. These responses point to the fact that the skin recognizes when its barrier is compromised, but does not necessarily distinguish what type of barrier (i.e. permeability, immune, UV, etc.) is compromised. The barrier repair response encompasses the whole spectrum of barriers: upregulating lipid synthesis, pigmentation, mobilization of the sentinel cells, antimicrobial peptides such as defensins and possibly the anti-oxidants such as vitamin E. The sebaceous gland is a source of vitamin E [33], and a relationship between stress and acne as shown recently [34] could reflect an increased supply of an antioxidant as well as defensin [35] to the skin surface. The responses of sweat glands, another source of antibiotic peptides [36] also remain under-investigated. Dissecting the signaling events in these complex response pathways will not only be useful to adapt appropriate strategies for drug delivery, but also to eliminate undesirable side effects such as immune responses or hyper-/hypo-pigmentation. These areas, so far neglected, should be exciting and profitable areas of study for the basic and applied scientist alike.

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